CLAIMS

We claim:

1. A method for monitoring activity of one or more enzymes comprising the steps of:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) one or more enzymes that add or remove a moiety to or from said one or more binding partner polypeptides or one or more tagged binding partner polypeptides;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of said moiety, wherein addition or removal of said moiety promotes binding of said one or more binding partner polypeptides with the corresponding one or more tagged binding partner polypeptides; under conditions which promote binding of said one or more binding partner polypeptides with said one or more tagged binding partners; and

B. detecting said binding, wherein detection of binding as a result of said mixing is indicative of enzyme activity.

2. The method of claim 1 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

3. The method of claim 1 wherein both said one or more tagged binding partner polypeptides and said one or more binding partner polypeptides comprise one or more sites for the addition or removal of a moiety.

4. The method of claim 1 wherein said one or more tagged binding partner polypeptides are tagged with one or more fluorescent molecules.

5. The method of claim 4 wherein said detecting comprises monitoring the rate of diffusion of said fluorescent molecule.

6. The method of claim 1 wherein the step of detecting binding further comprises adding one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

7. The method of claim 6 wherein said one or more detector molecules comprise a said first region selected from the group consisting of a coiled-coil, an antigen, an epitope, an antibody, a single chain antibody, an oligonucleotide, avidin and its analogues and derivatives, and streptavidin, its analogs and derivatives; and wherein said one or more detector molecules comprise a said second region selected from the group consisting of an enzyme, a radioisotope, a radionuclide, a fluorochrome, and a fluorescent protein.

8. The method of claim 1 wherein one or more detector molecules are pre-bound to the one or more tagged binding partner polypeptides.

9. The method of claim 1 wherein the tag on said one or more tagged binding partner polypeptides comprises one or more radioactive molecules.

10. The method of claim 9 wherein said detecting comprises monitoring the presence or absence of radioactivity.

11. The method of claim 1 wherein said one or more binding partner polypeptides of step (ii) are tagged.

12. The method of claim 11 wherein the tag on said one or more binding partner polypeptides of step (ii) and said one or more tagged binding partner polypeptides comprises one or more fluorescent molecules.

13. The method of claim 12 wherein said detecting comprises monitoring the presence or absence of fluorescent resonance energy transfer (FRET).

14. A method for monitoring activity of one or more enzymes comprising the steps of:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides; and

(iii) one or more enzymes that add or remove a moiety to or from said one or more binding partner polypeptides or one or more tagged binding partner polypeptides;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of said moiety, wherein addition or removal of said moiety promotes dissociation of said one or more binding partner polypeptides from the corresponding one or more tagged binding partner polypeptides, under conditions which promote dissociation of said one or more binding partner polypeptides from said one or more tagged binding partner polypeptides; and

B. detecting dissociation of said one or more binding partner polypeptides from said one or more tagged binding partner polypeptides, wherein detection of dissociation as a result of said mixing is indicative of enzyme activity.

15. The method of claim 14 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

16. The method of claim 14 wherein both said one or more tagged binding partner polypeptides and said one or more binding partner polypeptides comprise one or more sites for the addition or removal of a moiety.

17. The method of claim 14 wherein the tag on said one or more tagged binding partner polypeptides comprises one or more fluorescent molecules.

18. The method of claim 17 wherein said detecting comprises monitoring the rate of diffusion of said one or more fluorescent molecules.

19. The method of claim 14 wherein the step of detecting further comprises adding one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

20. The method of claim 19 wherein said one or more detector molecules comprise a said first region selected from the group consisting of a coiled-coil, an antigen, an epitope, an antibody, a single chain antibody, an oligonucleotide, avidin and its analogues and derivatives, and streptavidin, its analogs and derivatives; and wherein a said one or more detector molecules comprise said second region selected from the group consisting of an enzyme, a radioisotope, a radionuclide, a fluorochrome, and a fluorescent protein.

21. The method of claim 14 wherein one or more detector molecules are pre-bound to the one or more tagged binding partner polypeptides.

22. The method of claim 14 wherein the tag on said one or more tagged binding partner polypeptides comprises one or more radioactive molecules.

23. The method of claim 22 wherein said detecting comprises monitoring the presence or absence of radioactivity.

24. The method of claim 14 wherein said one or more binding partner polypeptides of step (ii) are tagged.

25. The method of claim 24 wherein the tag on said one or more binding partner polypeptides of step (ii) and on said one or more tagged binding partner polypeptides comprises one or more fluorescent molecules.

26. The method of claim 25 wherein detecting binding of said one or more binding partner polypeptides of step (ii) to said one or more tagged binding partner polypeptides comprises monitoring the presence or absence of fluorescent resonance energy transfer (FRET).

27. The method of claim 1 or 14 wherein said one or more sites comprise a sequence which directs modification by an enzyme selected from the group consisting of a kinase, a phosphatase, a UDP-N-acetylglucosamine-dolichyl-phosphate-N-acetylglucosamine phosphotransferase, an O-GlcNAc transferase, a glycylpeptide-N-tetradecanoyl transferase, a carbohydrate transferase, a ubiquitin activating enzyme El, a ubiquitin conjugating enzyme E2, a ubiquitin conjugating enzyme Ubc9, a ubiquitin protein ligase E3, a poly (ADP-ribose) polymerase, a fatty acyl transferase, and an NAD:Arginine ADP ribosyltransferase.

28. The method of claim 1, wherein said site promotes addition of a chemical moiety selected from the group consisting of a phosphate moiety (PO_4), a ubiquitin moiety, a glycosyl moiety, an ADP-ribosyl moiety, a fatty acid moiety, and a sentrin moiety.

29. The method of claim 14 wherein said site promotes addition of a chemical moiety selected from the group consisting of a phosphate moiety (PO_4), a ubiquitin moiety, a glycosyl moiety, an ADP-ribosyl moiety, a fatty acid moiety, and a sentrin moiety.

30. The method of claim 1, wherein said site promotes removal of a chemical moiety selected from the group consisting of a phosphate moiety (PO_4), a ubiquitin moiety, a glycosyl moiety, an ADP-ribosyl moiety, a fatty acid moiety, and a sentrin moiety.

31. The method of claim 14, wherein said site promotes removal of a chemical moiety selected from the group consisting of phosphate moiety (PO_4), a ubiquitin moiety, a glycosyl moiety, an ADP-ribosyl moiety, a fatty acid moiety, and a sentrin moiety.

32. The method of claim 1 or 14 wherein said tag on said one or more tagged binding partner polypeptides is selected from the group consisting of a coiled-coil, an antigen, an epitope, an antibody, a single chain antibody, a nucleic acid binding domain, a radioactive amino acid, a fluorescent molecule, a reporter enzyme, and biotin.

33. The method of claim 1 or 14 wherein said site is recombinant.

34. The method of claim 1 or 14 wherein said site is naturally occurring.

35. A kit comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of a moiety, wherein addition or removal of said moiety promotes binding of said one or more binding partner polypeptides with the corresponding one or more tagged binding partner polypeptides; and wherein said one or more polypeptides and said one or more tagged binding partner polypeptides bind in a manner dependent on modification of said site.

36. The kit of claim 35 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

37. The kit of claim 35 further comprising one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

38. The kit of claim 35 wherein said one or more binding partner polypeptides further comprise one or more tags.

39. A kit comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides; and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of a moiety, wherein addition or removal of said moiety promotes dissociation of said one or more binding partner polypeptides from the corresponding tagged binding partner polypeptides, wherein said one or more polypeptides and said one or more tagged binding partner polypeptides dissociate in a manner dependent on modification of said site.

40. The kit of claim 39 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

41. The kit of claim 39 further comprising one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

42. The kit of claim 39 wherein said one or more binding partner polypeptides further comprise one or more tags.

43. A composition comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of a moiety, wherein addition or removal of said moiety promotes binding of said one or more binding partner polypeptides with the corresponding one or more tagged binding partner polypeptides; wherein said one or more binding partner polypeptides and said one or more tagged binding partner polypeptides bind in a manner dependent on modification of said site.

44. The composition of claim 43 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

45. The composition of claim 43 further comprising one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

46. The composition of claim 43 wherein said one or more binding partner polypeptides further comprise one or more tags.

47. A composition comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of a moiety, wherein addition or removal of said moiety promotes dissociation of said one or more binding partner polypeptides from the corresponding one or more tagged binding partner polypeptides; wherein said one or more polypeptides and said one or more tagged binding partner polypeptides dissociate in a manner dependent on modification of said site.

48. The composition of claim 47 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

49. The composition of claim 47 further comprising one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

50. The composition of claim 47 wherein said one or more binding partner polypeptides further comprise one or more tags.

51. A method of screening for a candidate modulator of enzymatic activity comprising:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) one or more enzymes that adds or removes a moiety to or from said binding partner polypeptide or said one or more tagged binding partner polypeptides;

wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides comprise one or more sites for the addition or removal of said moiety, wherein addition or removal of said moiety promotes binding of said one or more binding partner polypeptides with the corresponding one or more tagged binding partner polypeptides; under conditions which promote binding of said one or more binding partner polypeptides and said one or more tagged binding partner polypeptides; and

B. detecting binding of said one or more binding partner polypeptides to said one or more tagged binding partner polypeptides in both the presence and absence of a candidate modulator of enzymatic activity, wherein detection of the amount binding in the presence of the candidate modulator that is lesser or greater as compared to the amount of binding in the absence of the candidate modulator indicates modulation of enzymatic activity by said candidate modulator.

52. The method of claim 51 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

53. A method of screening for a candidate modulator of enzymatic activity comprising:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) one or more enzymes that adds or removes a moiety to or from said polypeptide;

wherein one or more tagged binding partner polypeptides or one or more binding partner polypeptides comprise one or more sites for the addition or removal of said moiety, wherein addition or removal of said moiety promotes dissociation of said one or more binding partner polypeptides from the corresponding one or more tagged binding partner polypeptides, under conditions which promote dissociation of said one or more binding partner polypeptides from said one or more tagged binding partner partners; and

B. detecting dissociation of said one or more binding partner polypeptides from said one or more tagged binding partner polypeptides in both the presence and absence of a candidate modulator of enzymatic activity, wherein detection of the amount of dissociation in the presence of the candidate modulator that is lesser or greater as compared to the amount of dissociation in the absence of the candidate modulator indicates modulation of enzymatic activity by said candidate modulator.

54. The method of claim 53 wherein said one or more tagged binding partner polypeptides or said one or more binding partner polypeptides are immobilized on a solid support.

55. A method for monitoring activity of one or more protease enzymes comprising the steps of:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (1); and

(iii) one or more protease enzymes

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promotes binding of said one or more immobilized binding partner polypeptides with the corresponding one or more tagged binding partners; under conditions which promote binding of said one or more immobilized binding partner polypeptides with said one or more tagged binding partners; and

B. detecting said binding, wherein detection of binding as a result of said mixing is indicative of protease activity.

56. A method for monitoring activity of one or more protease enzymes comprising the steps of:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) one or more protease enzymes

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promotes dissociation of said one or more immobilized binding partner polypeptides from the corresponding one or more tagged binding partners; under conditions which promote dissociation of said one or more immobilized binding partner polypeptides from said one or more tagged binding partners; and

B. detecting said dissociation, wherein detection of dissociation as a result of said mixing is indicative of protease activity.

57. The method of claim 55 or 56 wherein the tag on said one or more tagged binding partner polypeptides comprises one or more fluorescent molecules.

58. The method of claim 57 wherein said detecting comprises monitoring the rate of diffusion of said fluorescent molecule.

59. The method of claim 55 or 56 wherein the step of detecting binding further comprises adding one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

60. The method of claim 59 wherein said one or more detector molecules comprise a said first region selected from the group consisting of a coiled-coil, an antigen, an antibody, an oligonucleotide, a single chain antibody, avidin and its analogues and derivatives, and streptavidin, its analogs and derivatives; and wherein said one or more detector molecules comprise a said second region selected from the group consisting of an enzyme, a radioisotope, a radionuclide, a fluorochrome, and a fluorescent protein.

61. The method of claim 55 or 56 wherein one or more detector molecules are pre-bound to said one or more tagged binding partner polypeptides.

62. The method of claim 55 or 56 wherein the tag on said one or more tagged binding partner polypeptides comprises one or more radioactive molecules.

63. The method of claim 62 wherein said detecting comprises monitoring the presence or absence of radioactivity.

64. The method of claim 55 or 56 wherein said one or more immobilized binding partner polypeptides are tagged.

65. The method of claim 64 wherein said one or more immobilized binding partner polypeptides comprises a tag, and the tag on said one or more immobilized binding partner polypeptides and said one or more tagged binding partner polypeptides comprises one or more fluorescent molecules.

66. The method of claim 65 wherein said detecting comprises monitoring the presence or absence of fluorescent resonance energy transfer (FRET).

67. A kit comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promotes binding of said one or more immobilized binding partner polypeptides with the corresponding one or more tagged binding partners; under conditions which promotes binding of said one or more immobilized binding partner polypeptides with said one or more tagged binding partners.

68. A kit comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promotes dissociation of said one or more immobilized binding partner polypeptides from the corresponding one or more tagged binding partners; under conditions which promote dissociation of said one or more immobilized binding partner polypeptides from said one or more tagged binding partners.

69. The kit of claim 67 or 68 further comprising one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

70. The kit of claim 67 or 68 wherein the one or more immobilized binding partner polypeptides further comprise one or more tags.

71. A composition comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promotes binding of said one or more immobilized binding partner polypeptides with the corresponding one or more tagged binding partners; under conditions which promotes binding of said one or more immobilized binding partner polypeptides with said one or more tagged binding partners.

72. A composition comprising:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) packaging materials;

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promotes dissociation of said one or more immobilized binding partner polypeptides from the corresponding one or more tagged binding partners; under conditions which promote dissociation of said one or more immobilized binding partner polypeptides from said one or more tagged binding partners.

73. The composition of claim 71 or 72 further comprising one or more detector molecules comprising a first region that associates with said one or more tagged binding partner polypeptides and a second region comprising one or more reporter molecules.

74. The composition of claim 71 or 72 wherein said one or more immobilized binding partner polypeptides further comprise one or more tags.

75. A method of screening for a candidate modulator of enzymatic activity comprising:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) one or more protease enzymes

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said

protease digestion promotes binding of said one or more immobilized binding partner polypeptides to the corresponding one or more tagged binding partners; under conditions which promote binding of said one or more immobilized binding partner polypeptides to said one or more tagged binding partners; and

B. detecting binding of said one or more immobilized binding partner polypeptides to said one or more tagged binding partner polypeptides in both the presence and absence of a candidate modulator of protease activity, wherein detection of the amount binding in the presence of the candidate modulator that is lesser or greater as compared to the amount of binding in the absence of the candidate modulator indicates modulation of protease activity by said candidate modulator.

76. A method of screening for a candidate modulator of enzymatic activity comprising:

A. mixing:

(i) one or more tagged binding partner polypeptides;

(ii) one or more immobilized binding partner polypeptides that correspond to said one or more tagged binding partner polypeptides of step (i); and

(iii) one or more protease enzymes

wherein said one or more tagged binding partner polypeptides or said one or more immobilized binding partner polypeptides is susceptible to protease digestion, wherein said protease digestion promote dissociation of said one or more immobilized binding partner polypeptides from the corresponding one or more tagged binding partners; under conditions which promotes dissociation of said one or more immobilized binding partner polypeptides from said one or more tagged binding partners; and

B. detecting dissociation of said one or more immobilized binding partner polypeptides from said one or more tagged binding partner polypeptides in both the presence and absence of a candidate modulator of protease activity, wherein detection of the amount dissociation in the presence of the candidate modulator that is lesser or greater as compared to the amount of dissociation in the absence of the candidate modulator indicates modulation of protease activity by said candidate modulator.